UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: 13-SEP-2016

SUBJECT: **Glyphosate.** Study summaries for genotoxicity studies

PC Code: 417300
Decision No.: 521124
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0057499

MRID No.: NA

DP Barcode: D435656
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: 41071-83-6; 38641-94-0; 70393-85-0; 114370-14-8; 40465-76-7; 69254-40-6; 34494-04-7; 70901-12-1
40 CFR: §180.364

FROM: Greg Akerman, Ph.D.
Risk Assessment Branch 3
Health Effects Division (HED) (7509P)

THROUGH: Kelly Lowe, Acting Branch Chief
Risk Assessment Branch 1 (RAB1)
Health Effects Division (HED; 7509P)

TO: Khue Nguyen, Risk Manager Reviewer
Neil Anderson, Risk Manager
Pesticide Registration Division (RD; 7508P)

I. CONCLUSIONS

HED has reviewed the genotoxicity studies identified during the systematic review of glyphosate for the October 2016 SAP. Summary reviews of the genotoxicity studies included in the Glyphosate Issue Paper are provided below.

II. ACTION REQUESTED

Please review glyphosate genotoxicity studies for glyphosate SAP in October.

Summary reviews of genotoxicity studies included in the Glyphosate Issue Paper

Akanuma, M. (1995). HR-001: Reverse Mutation Test. Kodaira Laboratories. The Institute of Environmental Toxicology, Tokyo, Japan. Laboratory Project ID: IET 94-0142. April 3, 1995. MRID 50017102. Unpublished.

In two independent trials of a reverse mutation assay in bacteria *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* WP2 *uvrA* were exposed glyphosate; 95.68% purity in water at concentrations of 200, 500, 1000, 2000 and 5000 µg/plate (+/- S9 activation) in preincubation trials. The S9 fraction was derived from the livers of Sprague Dawley male rats induced with phenobarbital/ ß-napthoflavone. Standard strain-specific mutagens served as positive controls.

A preliminary dose range finding test did not show any toxicity to any strain up to 5000 µg/plate with and without S9 mx. There were no appreciable increases (*i.e.* < 2-fold the number of revertants of the corresponding solvent controls) in the mean number of revertants/plate in any strain in both trials. The positive controls induced the appropriate response in all strains in the presence and absence of S9-activation. Glyphosate is therefore, not mutagenic in this test system.

Alvarez-Moya et al. (2014). Comparison of the in vivo and in vitro genotoxicity of glyphosate isopropylamine salt in three different organisms. Genetics and Molecular Biology 37 (1): 105-110.

In this publication, the comet assay was use to evaluate the potential genotoxicity of glyphosate in three different organisms. This review focuses only on the findings conducted with mammalian cells (human lymphocytes). Human lymphocytes were prepared from peripheral blood samples were obtained from a finger puncture. Cell viability was asses by trypan blue exclusion. Slides of human lymphocytes were exposed to glyphosate (96% purity) at concentrations of 0.0007, 0.007, 0.07 and 0.7 mM for 20 h. Approximately 50 cells or nuclei per slide and two slides per experimental condition were used for analysis. The authors report a significant increase in DNA tail length with all concentrations of glyphosate compared to the negative controls. No measurements of tail intensity were reported.

Review comments: In this study peripheral blood was collected using finger stick rather than venipuncture which is the preferred method to collect peripheral blood. Cells were washed with PBS and then stored at 4ºC for an indeterminate amount of time before the cells were exposed to glyphosate. Cells were then treated for 20 h at room temperature. The preferred metric for the comet assay is DNA tail intensity. If measured, these values were not reported. The authors observed the same amount of DNA damage across 2 orders of magnitude and the positive control yielded the same level of DNA damage as the different levels of glyphosate. Based on these limitations, extreme caution should be used when interpreting the findings in this study.

Bolognesi et al. (1997). Genotoxic activity of glyphosate and its technical formulation roundup. Journal of Agricultural and Food Chemistry, 45 (5): 1957-62.

In a published literature paper, the genotoxic potential of glyphosate and glyphosate formulation Roundup were evaluated using the in vitro sister chromatid exchange (SCE) assay, in vivo alkaline elution assay and in vivo micronucleus assay. In addition, 8-OhdG adducts were measured in the liver and kidney of mice treated with glyphosate and Roundup. This review focuses only on the findings for glyphosate technical. The glyphosate used in these studies had a reported purity of 99.9%.

In the SCE assay, blood samples from two female donors were cultured and stimulate with phytohemagglutinin (PHA). After the addition of bromodeoxyuridine, cultures were incubated in the dark. Test chemicals were added 24 h after PHA stimulation. Colecemid was added 2 h prior to analysis. At 72 h from the onset of culture, slides were prepared and at least 50 metaphases were evaluated. The authors reported a statistically significant increase in SCE/cell at 1, 3 and 6 mg/mL glyphosate

Swiss CD1 mice were used in all in vivo assays. For the alkaline elution assay, groups of three males were i.p. injected with a single dose of glyphosate (300 mg/kg). The pH of glyphosate was monitored and adjusted to 7.0 prior to treatment. Animals were sacrificed at 4 and 24 h after injection. Nuclei from liver and kidney were lysed on filters and eluted. The authors reported a significant increase in the elution rate constant after 4 hours of treatment in the kidney and liver, but not after 24 h following treatment.

Liver and kidneys from the animals treated in the alkaline elution assay were process to obtain nuclei to test for DNA oxidative damage.by 8-hydroxydeoxyguanosine (8-OHdG) quantification. The separation of 8-OHdG and normal deoxynucleosides was performed by HPLC. The levels of 8-OHdG are expressed as the number of 8-OHdG adducts per 10^5 dG bases. The authors reported a statistically significant ($p<005$) induction of 8-OHdG in the DNA from the liver in mice treated for 24 hr, but not at 8 h. No 8-OHdG induction was observed in the kidney at either time point for glyphosate.

In the micronucleus assay, 3 male mice were treated with two i.p. injections (24 hours apart) of glyphosate (300 mg/kg). Methyl methanesulfonate served as the positive control. Animals were sacrificed 6 and 24 h after the last injection. The bone marrow was harvested and bone marrow smears were prepared and the number of polychromatic erythrocytes (PCE) among 1000 erythrocytes were determined for each mouse. The results were expressed as the mean number of PCEs following the scoring of 1000 PCEs. The authors reported a statistically significant ($p<0.05$) in the frequency of micronucleated cells at 24 h, but not 6 hr after ip injection with 300 mg/kg (2 x150 mg/kg) glyphosate. The positive control induced the appropriate response.

Reviewer Comments: The information provided in the materials and methods section is very limited. In the SCE assay, the SCE baseline appeared to be abnormally low. The authors claim a dose-response effect in the SCE assay; however, no statistics were reported to support that claim and the data were only presented in graph form with no error bars. Only 3 animals/dose

were used in the micronucleus study which is a very small N for this assay. There is no indication whether or not the slides were coded and if the scorers were blind to treatment.

Collander R.D. (1996). Glyphosate Acid: An Evaluation of Mutagenic Potential Using *S. typhimurium* and *E. coli*. Central Toxicology Laboratory, Cheshire, UK. Laboratory Project ID: CTL/P/4874 Study No. YV3611. February 16, 1996. MRID 44320617. Unpublished.

In two separate trials of a reverse mutation assay in bacteria *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* WP2 and WP2 *uvrA* were exposed to glyphosate acid; 95.6 % purity in dimethylsulfoxide (DMSO) at concentrations of 100, 200, 500, 1000, 2500 and 5000 µg/plate (+/- S9 activation). Glyphosate acid was initially tested using the plate incorporation method and subsequently re-tested using the preincubation method. The S9 fraction was derived from the livers of Sprague Dawley male rats induced with phenobarbital/ ß-napthoflavone. Standard strain-specific mutagens served as positive controls.

The mean number of revertants was less than 2-fold the number of revertants of the corresponding controls with and without S9-activation at all test material concentrations. The positive controls induced the appropriate response in all strains in the presence and absence of S9-activation. Glyphosate acid is therefore, not mutagenic in this test system.

Chruscielska, K. et al. 2000. Glyphosate Evaluation of chronic activity and possible far-reaching effects. Part 2. Studies on mutagenic activity. Pestycydy, 2000, (3-4), 21-25. Published.

In a published literature study, *Salmonella typhimurium* strains TA97a, TA98, TA100, and TA102 were exposed to glyphosate (purity not provided) at concentrations of 250 to 2000 µg/plate with and without S9 activation. The S9 fraction was reported to be derived from the microsomal fraction of rat livers induced by Aroclor. Glyphosate was reported to toxic to the tester strains at 4000 µg/plate. No increase (<2-fold increase compared to corresponding negative controls) in revertant colonies was reported at any test concentration in the presence or absence of S9 activation.

The study also evaluated glyphosate using the micronucleus test to detect potential clastogenic effects in bone marrow cells. Six C3H male mice were i.p. injected with glyphosate (purity not reported) with a single dose of 300 mg/kg. Bone marrow was harvest at 24, 48 and 72 hours. Endoxan (75 mg/kg) was used as the positive control. 1000 polychromatic erythrocytes from each animal were analyzed. The authors concluded that that glyphosate did not reveal genotoxic activity in the micronucleus test in mouse bone marrow cells.

Durward, R. (2006). Technical Glyphosate: Micronucleus Test in the Mouse. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire DE72 2GD, UK, Study No. 2060/014. February 8, 2006. MRID 49957411. Unpublished.

In a bone marrow micronucleus assay, adult male Crl: CD-1® (ICR) mice were treated once via intraperitoneal injection with glyphosate (95.7% purity) in phosphate buffered saline (PBS). Doses of 0, 150, 300 and 600 mg/kg were administered to groups of 7 male mice. One group of mice from each dose level was killed by cervical dislocation 24 hours following treatment and another group dosed with test material at 600 mg/kg was killed after 48 hours. Bone marrow cells were harvested at 24 hours and 48 hours following dosing and scored for micronucleated polychromatic erythrocytes (MPCEs) and the ratio of polychromatic to normochromatic erythrocytes (PCE: NCE). Cyclophosphamide (50 mg/kg) served as the positive control.

Based on the findings from the range-finding toxicity study, no substantial differences in the toxicological response of the male or female mice up to 1000 mg/kg. In the main study, there were no premature deaths seen in any of the dose groups. There were small but statistically significant reductions in the PCE: NCE ratio for the 24 hour high-dose males. The response however was very modest, within historical control range for vehicle control animals and did not include any individual animal values that would not be acceptable for vehicle control animals. The positive control showed a marked increase in the incidence of micronucleated polychromatic erythrocytes hence confirming the sensitivity of the system to the known mutagenic activity of cyclophosphamide under the conditions of the test. There was no significant increase in the frequency of MPCEs in any treatment group at either harvest time.

Flowers and Kier (1978). Salmonella mutagenicity assay (Monsanto Company, Environmental Health Laboratory; June 16, 1978; Test material-Glyphosate; Sample No. 04; Test No. LF-78-161). MRID 00078620. Unpublished.

The study design followed the methods and materials described by Ames et al. in Mutation Research (1975) Vol. 31; pp. 347-364. Salmonella strains used in the plate incorporation assays were TA98, TA100, TA1535 and TA1537. The concentration of 98.4% glyphosate were 0.1, 0.4, 2.0, 30.0, 100 and 1000 µg/plate. Testing was conducted with and without S9 activation. The proper positive controls were used. Glyphosate was not mutagenic toward and Salmonella strains under the test conditions.

Fox, V. (1998). Glyphosate acid: In vitro cytogenetic assay in human lymphocytes. Central Toxicology Laboratory, Cheshire, UK. Report CTL/P/6050. October 29, 1998. MRID 49961803. Unpublished.

The clastogenic potential of glyphosate acid was evaluated in an *in vitro* cytogenetic assay using human lymphocytes donated from two donors. The study was conducted according to OECD guideline 473. Duplicate cultures (from donors 1 and 2) were exposed to the glyphosate (95.6% purity) at concentrations of 100, 750 and 1250 µg/mL with and without S9- activation for 68 hours. Additionally, duplicate cultures from donor 2 were also exposed to 1250 µg/mL with and without S9 for 92 hours. The highest test concentration was selected based on significant reductions in pH of the culture medium at 1500 and 2000 µg/mL (pH 6.73 and 6.40, respectively versus pH 7.51 for solvent control). Mitomycin C and cyclophosphamide served as the positive controls. Approximately 2 hours before harvesting, cultures were treated with colcemid. All slides were coded prior to analysis and 100 cells in metaphase were analyzed from each culture

for chromosomal aberrations (including and excluding gap-only type aberrations). The mitotic index was determined by examining 1000 lymphocytes per culture.

Minor reductions (33-37%) reductions in the mitotic indices were observed at the highest test concentrations. No statistically significant increases in the percentage of cells with chromosomal aberrations were observed from either donor at any glyphosate test concentration with or without S9 activation at the 68 hour sampling time. Additionally, no statistically significant increase in chromosomal aberrations were seen in cells exposed to 1250 µg/mL with and without S9 for 92 hours. The positive controls induced the appropriate response in the presence and absence of S9-activation. Glyphosate is considered to be negative in this test system.

Fox, V., and Mackay, J.M. (1996). Glyphosate Acid: Mouse Bone Marrow Micronucleus Test. Central Toxicology Laboratory. Study No: SM0796. March 21, 1996. MRID 44320619. Unpublished.

In a bone marrow micronucleus assay, 5 adult male and female CD-1 mice per dose were treated with a single oral dose of 5000 mg/kg of glyphosate acid (95.6% purity). Phase I of the study involved the determination of a maximum tolerated dose (MTD), based on patterns of lethalities or severe toxicity observed over a four-day observation period following treatment. In phase II, male and female animals were weighed and given a single oral dose of physiological saline, cyclophosphamide (65 mg/kg) or glyphosate acid at a dose level of 5000 mg/kg. Bone marrow smears were prepared 24 and 48 hours after dosing for the vehicle control and glyphosate acid treated animals and 24 hours after dosing for the cyclophosphamide treated animals.

For Phase I, no clinical signs or lethalities were observed over a four day observation period, at the limit dose level of 5000 mg/kg which was selected to represent the maximum tolerated dose for both males and females. For Phase II, no adverse reactions to treatment were observed for either males or females dosed with glyphosate acid at the limit dose of 5000 mg/kg. No statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were observed in either males or females at either sampling time investigated. The test system positive control, cyclophosphamide, induced statistically and biologically significant increases in the frequency of micronucleated polychromatic erythrocytes in both male and female mice at the 24 hour sampling time.

Hornarvar, N. (2008). Technical Glyphosate: Micronucleus Test of the Mouse. RCC, Cytotest Cell Research GmbH (RCC-CCR0, In den Leppsteinswiesen 19, 64380 Rossdorf, Germany, Study No. 1158500. June 9, 2008. MRID 49961802. Unpublished.

In a bone marrow micronucleus assay, adult male NMRI mice were treated once orally with glyphosate (99.1% purity) in 0.5% Carboxymethylcellulose (CMC). Doses of 0, 500, 1000 and 2000 mg/kg were administered to groups of 6 male mice. The animals of all dose groups were examined for acute toxicity symptoms at intervals of around 1 h, 2 – 4 h, 6 h (high dose group animals, 2000 mg/kg were not observed at 6 h), 24 h and 48 h after administration of the test item. Bone marrow cells were harvested at 24 hours and 48 hours following dosing and scored

for micronucleated polychromatic erythrocytes (MPCEs) and the ratio of polychromatic to normochromatic erythrocytes (PCE: NCE). Cyclophosphamide (40 mg/kg) served as the positive control (6 mice).

Based on the findings from the range-finding toxicity study, no toxic responses were observed in male or female mice up to 2000 mg/kg. In the main study, there were no premature deaths or clinical signs observed in any of the dose groups. There were no reductions in the PCE: NCE ratios 24 or 48 hours post dose. The positive control responded appropriately. There was no significant increase in the frequency of MPCEs in any treatment group at either harvest time.

Jensen, JC, (1991a). Mutagenicity test: Ames Salmonella Assay with Glyphosate, Batch 206-JaK-25-1. Scantox A/S, Lemvig, Denmark. Laboratory No. 12323. October 9, 1991. MRID 49961502. Unpublished.

In two independent trials of a reverse mutation assay in bacteria *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 were exposed glyphosate; 98.6% purity in water at concentrations of 310, 630, 1300, 2500 and 5000 µg/plate with S9 activation and 160, 310, 630, 1300 and 2500 µg/plate without S9 mix. The S9 fraction was reported to be derived from the microsomal fraction of SPF Wistar rat livers induced by Aroclor 1254. A preliminary toxicity test was performed with TA98 strain at concentrations of glyphosate from 560 to 5000 µg/plate. The first test trial was conducted with all tester strains using the plate incorporation assay followed by a second trial using the preincubation method. Standard strain-specific mutagens served as positive controls.

The preliminary toxicity test showed evidence of toxicity at 5000 µg/plate in the absence of S9 mix, therefore, 2500 µg/plate was selected as the highest test concentration for main assays (– S9). No statistically significant increases in the number of revertants were seen with any of the strains in the presence or absence of S9-activation. The positive controls induced the appropriate response in all strains in the presence and absence of S9 mix. Glyphosate is not mutagenic in this test system.

Jensen, J.C. (1991b). Mutagenicity test: In vitro mammalian cell gene mutation test with glyphosate, batch 206-JaK-25-1. Scantox A/S, Lemvig, Denmark. Laboratory No. 12325. October 9, 1991. MRID 49961504. Unpublished.

An in vitro cell gene mutation assay was conducted with mouse lymphoma cells L5178Y according to OECD guideline 476. In two independent trials, L5718Y cells were exposed to glyphosate (98.6% pure) dissolved in culture medium at concentrations of 630, 1300, 2500 and 5000 µg/ml in the absence of S9-activation and 520, 1000, 2100 and 4200 µg/ml in the presence of S9-mix. The S9 fraction was obtained from the liver of Wistar rats induced with Aroclor 1254. The glyphosate concentrations tested in the mutagenicity test were based on the findings of a preliminary toxicity test.

No substantial reduction in cloning efficiency was seen at any of the test concentrations of glyphosate. Glyphosate did not induce a statistically significant or reproducible increase in mutation frequency compared to the negative controls. The mutation frequency for the control cultures were in the expected range and the positive control (DMBA) induced a positive response in the test system. Glyphosate is considered to be negative in this test system.

Jensen, J.C. (1991c). Mutagenicity Test: Micronucleaus test with Glyphosate, batch 206-JaK-25-1. Scantox A/S. Laboratory No.: 12324. December 9, 1991. MRID 49961503. Unpublished.

In a bone marrow micronucleus assay, adult male NMRI mice were treated once orally with glyphosate (99.1% purity) in 0.5% Carboxymethylcellulose (CMC). A single oral dose of 5 mg/kg were administered to groups of three groups of male and female mice. A negative control was dosed orally with an equivalent volume of the vehicle. Bone marrow cells were harvested at 24, 48 and 72hours following dosing and scored for micronucleated polychromatic erythrocytes (MPCEs) and the ratio of polychromatic to normochromatic erythrocytes (PCE: NCE). Cyclophosphamide (30 mg/kg) served as the positive control (5 mice).

Based on the findings from the range-finding toxicity study, no toxic responses were observed in male or female mice up to 5 mg/kg. In the main study, there were no premature deaths or clinical signs observed in any of the dose groups. There were no reductions in the PCE: NCE ratios 24 or 48 hours post dose; the range was 37.7 to 42.2%. The positive control responded appropriately. There was no significant increase in the frequency of MPCEs in any treatment group at either harvest time.

Li, A.P. (1983a). CHO/HGPRT Gene mutation assay with glyphosate. Environmental Health Laboratory, St. Louis, MO. October 20, 1983. MRID 00155528. Unpublished.
Glyphosate (98.7% purity) was tested for potential mammalian mutagenicity in culture Chinese hamster ovary (CHO) cells at concentrations of 3-25 mg/mL in the presence and absence of S9 activation. Aroclor 1254-induced rat liver was the source of the S9. The positive controls were benzo(a)pyrene and ethyl methane sulfonate with and without S9, respectively. A previous range finding study showed that approximately 90% cell death was observed between 20 and 25 mg/mL glyphosate. Glyphosate in this study was cytotoxic at concentrations above 10 mg/mL. No significant increase in mutations at the HGPRT gene locus were observed at any test concentration. Glyphosate was negative in this test system.

Li, A.P. (1993b). In vivo bone marrow cytogenetic study of glyphosate in Sprague Dawley rats. Environmental Health Laboratory, St. Louis, MO. October 20, 1983. MRID 00132683. Unpublished.

In an in vivo chromosomal aberration assay, Six male and six female Sprague Dawley rats were i.p. injected with 1000 mg/kg glyphosate (purity 98.7%) and bone marrow was harvested after 6, 12 or 24 hours. The dose selected was considered to be the maximum soluble concentration for the volume to be injected. Cyclophosphamide was used as a positive control. Approximately 50 mitotic cells (300 cells per treatment) were scored for chromosomal aberrations. No statistically

significant increases in chromosomal aberrations were reported in either sex at any time point. The positive controls responded appropriately in the test system.

Li and Long (1988) An evaluation of the genotoxic potential of glyphosate. Fundam Appl Toxicol 10(3): 537-546.

This paper presents genotoxicity findings from a number of different assays. Most of the findings are summarized as individual studies in this document. This review will focus on the rat hepatocyte unscheduled DNA synthesis (UDS) assay. In this assay, non-induced primary rat hepatocytes from an adult male Fischer 344 rat were used to evaluate the potential for glyphosate to induce unscheduled DNA synthesis in mammalian cells. Benzo(a)pyrene served as a positive control. The cells were cultured on coverslips (disks) for 2 h. The cells were treated with glyphosate (purity 98%) at 20-2000 µg/disk in the presence of tritiated thymidine for 18-20 h. Nuclear grains were scored and UDS was quantified as net grains/nucleus. No statistically significant increases in net grains/nucleus were observed for any test concentration of glyphosate.

Koller, V., et al. (2012). Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. Arch Toxicol. 86: 805-813.

In a published literature study, TR146 cells (a human carinoma cell line of buccal epithelial origin) were exposed to glyphosate (95% purity) or the glyphosate formulation Roundup (a 450 g/L glyphosate acid) for 20 minutes. This review focuses on the finding for glyphosate technical only. Single cell gel electrophoresis (comet) and in vitro micronuclei (MN) assays were performed on the cells following treatment.

In the comet assay, TR146 cells were exposed to glyphosate at 10- 2000 µg/mL for 20 minutes. The cells were processed and 50 cells were evaluated for each condition for comet formation. The authors reported a statistically significant ($p<0.05$) increase in DNA tail intensity at concentrations of 20 µg/mL and higher. No positive controls were used in this assay.

In the MN assay, TR146 cells were exposed to 10, 15 or 20 µg/mL glyphosate. Following the 20 minute exposure to glyphosate, cells were washed and culture in medium containing cytochalasin B. The total incubation time was 48 h. The cells were processed and the total number of micronuclei in binucleated cells and number of bincucleated cells with micronuclei were determined. Additionally the number of nuclear buds (NB), nucleoplasmic bridges (NPB), apoptotic and necrotic cells were calculated. For each culture, more than 1000 binucleated cells were evaluated. Mitomycin C served as the positive control. The authors reported a significant increase in micronucleated cells at all test concentrations evaluated. Significant increases were also reported for NB and NPB at all concentrations. A statistically significant increase in apoptotic and necrotic cells were reported at 20 µg/mL. The positive control yielded the appropriate response.

Reviewer comments: The cell line used in this study is derived from a metastatic tumor of buccal epithelial origin and are not primary buccal cells. Caution should be used when interpreting genotoxicity results from tumor cell lines in cases where the cell line has not been well-characterized regarding DNA damage response, repair capacity and genomic stability which may impact the interpretation of the results. Also, there is no indication that pH was monitored or adjusted in these assays. Glyphosate has been shown to decrease the pH of some culture media resulting in false positives in genotoxicity assays. Furthermore, there is no indication that the samples were coded in either assay and scorers were blind to treatment. These limitations may impact the interpretation and significance of the findings.

Lioi M.B., et al. (1998a). Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro. Mutation Research- Fundamental and Molecular Mechanisms of Mutagenesis. 403 (1-2), 13-20.

In a published literature study, the genotoxicity of three pesticides (glyphosate, vinclozolin and DPX-E9636; ≥98% purity) were evaluated in cultured bovine lymphocytes in vitro using chromosomal aberration (CA) and sister chromatid exchange (SCE) assays. In addition, glucose 6-phosphate dehydrogenase (G6PD) activity was measured to assess the redox state of the cells following treatment. Lymphocytes were isolated from the peripheral blood drawn from three unrelated, healthy cows. The methods and results described in this summary focus only on glyphosate. The lymphocytes were cultured and exposed to glyphosate at concentrations of 17, 85 or 170 µM (in water) immediately following pokeweed stimulation. Cells were exposed for a total of 72 hours. For the CA analysis, cells were treated with colcemid two hours prior to harvesting. For the SCE analysis, BrdU was added 30 hours prior to harvesting and colcemid was added during the final 2 hours. Fifty metaphases were prepared from each subject for each test concentration. Slides were blindly coded for CAs and SCEs. The mitotic index was determined by counting at least 10000 cells per treatment.

CA Results: The authors report a statistically significant ($p<0.05$) increase in the percentage of aberrant cells and aberration frequency at all test concentrations of glyphosate. The mean percentage of aberrant cells was 12.7 ± 3.1, 21.3 ± 4.6, 27.3 ± 5.7 for 17, 58 and 170 µM, respectively, versus 4.0 ± 2.0 for the controls. The mean aberration frequency was 19.3 ± 4.2, 31.3 ± 6.1, 38.7 ± 7.0 at 17, 58 and 170 µM, respectively, versus 4.7 ±1.2 for the controls. A significant reduction in mitotic index was reported at all test concentrations.

SCE Results: A statistically significant ($p<0.05$), but non-concentration dependent increase in SCE/cell was reported for each test concentration of glyphosate. The mean SCE/cell values were 9.4 ± 2.1, 10.9 ± 1.6, 8.5 ± 1.6 for 17, 85 and 170 µM, respectively compared to 5.2 ± 0.6 for the controls.

Additionally, the authors reported that a significant enhancement of G6PD activity was observed in all treated lymphocyte cultures compared to the controls.

Reviewer's comments: The cultured bovine lymphocytes in this study were exposed for 72 h in the presence of glyphosate and analyzed CAs and SCEs, which is too long for analyzing one

round of mitosis. Data were pooled from 3 cows rather than establishing a mean ± SD for each animal and then taking a mean of the means. No positive controls were used in the study. Also it is unusual that nearly the same level of effect for aberration frequency and % of cells with aberrations was found for the same concentrations of glyphosate, vinclozolin, and DPX-E9636. These limitations/observations lessen the impact of the reported findings.

Lioi M.B., et al. (1998b). Cytogenetic Damage and Induction of Pro-Oxidant State in Human Lymphocytes Exposed In Vitro to Gliphosate, Vinclozolin, Atrazine, and DPX-E9636. Environmental and Molecular Mutagenesis 32: 39-46.

In a published literature study, the genotoxicity of four pesticides (glyphosate, vinclozolin, atrazine, and DPX-E9636; ≥98% purity) were evaluated in cultured human lymphocytes in vitro using chromosomal aberration (CA) and sister chromatid exchange (SCE) assays. In addition, glucose 6-phosphate dehydrogenase (G6PD) activity was measured to assess the redox state of the cells following treatment. Lymphocytes were isolated from the peripheral blood drawn from three unrelated, healthy human donors. The methods and results described in this summary focus only on glyphosate. The lymphocytes were cultured and exposed to glyphosate at concentrations of 5.0, 8.5, 17.0 or 51.0 μM (in water) immediately following phytohemagglutinin stimulation. Cells were exposed to pesticides for a total of 72 hours. For the CA analysis, cells were treated with colcemid two hours prior to harvesting. For the SCE analysis, BrdU was added 30 hours prior to harvesting and colcemid was added during the final 2 hours. Fifty metaphases were prepared from each subject for each of the four test concentrations. Slides were blindly coded for CAs and SCEs. The mitotic index was determined by counting at least 10000 cells per treatment.

CA Results: The authors reported a statistically significant ($p<0.05$) increase in the percentage of aberrant cells and aberration frequency at ≥8.5 μM glyphosate. The mean percentage of aberrant cells was 3.3 ±4.2, 6.7 ± 3.1, 23.3 ± 3.1, 27.3 ± 5.0 for 5, 8.5, 17 and 51 μM, respectively, versus 0.7 ± 1.2 for the controls. The mean aberration frequency was 3.3 ± 4.2, 9.3 ± 3.1, 30.0 ± 2.0, 34.7 ± 1.2 at 5, 8.5, 17 and 51 μM, respectively, versus 0.7+1.2 for the controls. The mitotic index was not significantly reduced.

SCE Results: A statistically significant ($p<0.05$), but non-concentration dependent increase in SCE/cell was reported at ≥8.5 μM glyphosate. The mean SCE/cell values were 2.3 ± 0.6, 3.6 ± 0.8, 5.3 ±0.3, 4.9 ±1.3 for 5, 8.5, 17 and 51 μM, respectively, compared to 1.9 ± 0.9 for the controls.

Additionally, the authors reported that a significant enhancement of G6PD activity was observed at ≥8.5 μM glyphosate compared to the controls.

Reviewer comments: The cultured human lymphocytes in this study were exposed for 72 h in the presence of glyphosate and analyzed CAs and SCEs, which is too long for analyzing one round of mitosis. . Data were pooled from 3 donors rather than establishing a mean ± SD for each donor. No positive control chemicals were used in the study. These limitations may impact interpretation of the reported findings.

Majeska, J (1982a). Mutagenicity evaluation in Salmonella Typhimurium. Environmental Health Center, Farmington, CT. Report Number T-10847. January 19, 1982. MRID 00126612.

In two independent trials of a reverse mutation assay in bacteria *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed glyphosate (90% purity) in water at concentrations of 0.12-10 mg/plate (-S9) and 0.56-15 mg/plate (+ S9 activation). The S9 was derived from Aroclor 1254-induced rat livers. Standard strain-specific mutagens served as positive controls.

There were no appreciable increase in the mean number of revertants/plate in any strain in both trials. The positive controls induced the appropriate response in all strains in the presence and absence of S9-activation. Glyphosate is therefore, not mutagenic in this test system.

Majeska, J. (1982b). Morphological transformation of Balb/3T3 cells. Report No. T-10884. MRID 00126616. Unpublished.

In a cell transformation assay, Balb/3T3 cells were exposed to glyphosate trimesium salt (90% purity) at concentrations of 0.313, 0.625, 1.25, 2.5 and 5.0 mg/mL. Glyphosate did not induce morphological transformation in this test system.

Majeska, J. (1982c). Mutagenicity evaluation of bone marrow cytogenetic analysis in rats. Report No. T10884. MRID 00126611. Unpublished.

In an in vivo chromosomal aberration assay, 8 Sprague Dawley rats (per condition) were administered glyphosate (58.5% purity) at doses of 21, 68 and 188 mg/kg by oral gavage and sacrificed bone marrow cells harvested at 6 h, 12 h, 24 h and 51 h after initial dosing. The high dose was selected to approximate 1/3 of the LD50. Three hours prior to sacrifice the animals were i.p. injected with colchicine. Slides were prepared and 100 spreads were read for each animal when possible. Cyclophosphamide served as the positive control. No spastically significant increases in chromosomal aberrations were observed at any dose or time point. The positive control responded appropriately in this test system.

Majeska, J (1985a). Mutagenicity evaluation in Salmonella Typhimurium. Environmental Health Center, Farmington, CT. Report Number T-12600. September 25, 1985. MRID 0015527. Unpublished.

In two independent trials of a reverse mutation assay in bacteria *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 were exposed glyphosate trimesium salt (55.6% purity) at concentrations 0.05 to 50 µg/mL with and without S9 activation. The S9 was derived from Aroclor 1254-induced rat livers. Standard strain-specific mutagens served as positive controls.

There were no appreciable increase in the mean number of revertants/plate in any strain in both trials. The positive controls induced the appropriate response in all strains in the presence and absence of S9-activation. Glyphosate is therefore, not mutagenic in this test system.

Majeska, J (1985b). <Mutagenicity evaluation in mouse lymphoma multiple endpoint test forward mutation assay. Environmental Health Center. Report No. T12661. December 12, 1985. MRID 00155528. Unpublished.

Glyphosate trimesium salt (purity 55.6%) was evaluated for its ability to induce forward mutations at the thymidine kinase (TK) locus in L5178Y mouse lymphoma cells. The study was conducted in the presence and absence of S9 activation. The source of the S9 was Aroclor 1254-induced rat livers.

In an initial range finding study, glyphosate trimesium salt was shown to be toxic at concentrations of 5.0 µl/ml and higher. The addition of S9 did not alter the toxicity at those concentrations. Ethyl methanesulfonate and N-nitrosodimethylamine were used as positive controls. In initial tests, glyphosate trimesium salt induced reproducible increases in mutation frequencies at concentrations above 3.0 µl/ml without S9 and at 2.5 µl/ml and above in the presence of S9. At these concentrations significant reductions in the pH (up to 2.0 pH units) of the test medium were observed.

When glyphosate trimesium salt was retested when the pH of the test concentrations were adjusted to 7.2-7.5, toxicity was reduced and there was there was no increase in mutant frequency in the presence or absence of S9 activation. Glyphosate trimesium salt is not mutagenic under proper testing conditions (i.e. appropriate pH and osmolality) in this test system.

Majeska, J (1985c). Mutagenicity evaluation in Chinese hamster ovary cytogenetic assay. Environmental Health Center, Farmington, CT. Report Number T-12663/SC-0224. December 18, 1985. MRID 00155530. Unpublished.

Glyphosate trimesium salt (55.6% purity) was evaluated for its potential to induce chromosomal aberrations in Chinese hamster ovary (CHO) cells, in vitro. Glyphosate trimesium salt was tested at concentrations of 4.0- 10.0 ul/ml with and without S9 activation. At higher concentrations, the osmolality increased and was toxic to the cells. The pH was adjusted to 7.4-7.6 prior to treatment. Mitomycin C and cyclophosphamide served as the positive controls with and without S9, respectively. The source of the S9 was Arocolor 1254-induced rat livers. Approximately 14 h after the initiation of treatment, the cells were treated with colcimid and incubated for an additional 2 hours. No statistically significant increases in chromosomal aberrations were reported when tested under proper culture conditions (i.e. appropriate pH and osmolality) in this test system.

Majeska, J (1987). Mutagenic evaluation in bone marrow micronucleus. Environmental Health Center, Farmington, CT. Study Report No. T12689/SC-0024. April 23, 1987. MRID 40214004. Unpublished.

In a bone marrow micronucleus assay, CD1 mice were treated with glyphosate (53.3% purity) in water by oral gavage. The doses for males were 700, 900 and 1100 mg/kg and the doses for females were 400, 600 and 800 mg/kg. Bone marrow cells were harvested at 24, 48 and 72 h following dosing and scored for micronucleated polychromatic erythrocytes (MPCEs) and the ratio of polychromatic to normochromatic erythrocytes (PCE: NCE). Cyclophosphamide served as the positive control.

There were no effect of glyphosate technical on micronucleus formation on mice bone marrow erythrocytes when compared to the control. The positive control responded appropriately. There were no dose related or time related increases in the frequency of MPCEs in any treatment group or sex.

Manas, F., et al (2009). Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests. Environmental Toxicology and Pharmacology. 28: 37-41.

In this literature study, glyphosate (96% purity) was evaluated for its genotoxic potential in three different test systems: an in vitro single cell gel electrophoresis (comet) assay performed in Hep-2 cells, an in vitro chromosomal aberration (CA) test in human lymphocytes and an in vivo bone marrow micronucleus (MN) test in mice.

In the comet assay, Hep-2 cells were treated for 4 h with 3.0, 4.5, 6.0, 7.5, 9.0, 12.0 and 15.0 mM glyphosate. Mitomycin C was used as a positive control. Viability was reported to be lower than 80% at concentrations above 7.5 mM. The authors reported a statistically significant ($p<0.01$) increase in tail intensity, tail length and tail moment at all concentrations of glyphosate tested. The positive control induced significant increases in tail intensity, length and moment.

In the CA assay, blood samples from 6 human donors (3 male/3 female) were cultured. The cultured lymphocytes were exposed to glyphosate at concentrations of 0.2, 1.2 or 6.0 mM for 48 h. The pH of the culture medium was adjusted to 7.2-7.4 after the addition of glyphosate. Mitomycin C served as the positive control. The mitotic index was calculated by examining 2000 cells/culture. One hundred metaphases/group was analyzed for CA. Slides were scored blind by two observers. No statistically significant difference in the mitotic indices were seen at any test concentration. No statistically significant increases in CA were observed at any test concentration compared to the controls. The positive control induced the appropriate response.

In the MN assay, groups of 5 male and female Balb C mice were i.p. injected with 50, 100 or 200 mg/kg. All doses were repeated after 24 h. Cyclophosphamide served as the positive control. Animals were sacrificed 24 h after the last dose and the bone marrow was harvested. Approximately 1000 erythrocytes (described only as "erythrocytes" in the materials and methods section) were scored for the presence of micronuclei. The ratio of polychromatic erythrocytes/normochromatic erythrocytes was calculated to assess bone marrow toxicity. The

authors reported a statistically significant ($p<0.05$) increase in the frequency of micronucleated cells at 200 mg/kg (13.0 ± 3.5 vs. 3.8 ± 0.8 in the negative controls), but not at lower doses. The positive control induced the appropriate response.

This study also measured markers of oxidative stress (thiobarbituric acid reactive substances (TBARs) levels, superoxide dismutase (SOD) and catalase (CAT) activities) in mice (5/dose) injected via i.p. with 400 mg/kg (single dose) following 1 or 2 h exposures. The liver, kidney, lung and heart were evaluated. The only statistically significant ($p<0.05$) increase in any of the marker was an increase in CAT activity was reported at 1 h in the liver; however it was not significant at 2 h and no other significant changes in the oxidative stress markers were observed.

Reviewer comments: The comet assay was conducted in Hep-2 cells. These cells are apparently a derivation of HeLa cervical carcinoma cell line. It is not clear if these cells have been well-characterized regarding DNA damage response, repair capacity and genomic stability which may impact the interpretation of the results. Also, glyphosate has been shown to decrease the pH of some culture media and affect cell viability and induce genetic damage. Although the authors state that the pH was adjusted to 7.2-7.4 following addition of glyphosate to the test medium in the CA assay, there no mention that the pH was adjusted following addition of glyphosate in the comet assay. Similarly, in the CA assay the authors state that the slides were coded and the scoring was performed blind. There is no indication in the MN assay that the scorers were blind to treatment, which may bias the findings. These limitations should be considered when interpreting the results from this paper.

Manas, F., et al. (2013). Oxidative stress and comet assay in tissues of mice administered glyphosate and ampa in drinking water for 14 days. BAG Journal of basic and applied genetics *24*, 67-75.

In this published study, Balb C mice were exposed to glyphosate (96% purity) at 40 or 400 mg/kg/day in drinking water for 14 days. At the end of 14 days, peripheral blood was collected from the tail vein and heart, lung, liver and kidney organs were collected. Blood and liver were evaluated for DNA damage using the comet assay. Additionally, markers of oxidative stress (i.e. levels of thiobarbituric acid reactive substances (TBARs); quantified superoxide dismutase (SOD) and catalase (CAT) activity in liver, kidney, lung and heart. The authors reported that exposure to glyphosate at 400 mg/kg induced a statistically significant ($p <0.05$) decrease of SOD activity in heart and an increase in CAT activity in kidney. In the comet assay there were statistically significant differences in all the treatments and tissues studied in comparison to control animals ($p\leq 0.01$). The DNA effects did not appear to be associated with oxidative stress.

Reviewer comments: No positive control was used in this study. Also there is no mention that the scorers were blind to treatment. There is some uncertainty regarding how the data are presented in Table 1. The reader must assume which values are means and which values are SEMs, given that the values are not presented the same way in each row. Tail intensity is the most meaningful metric when evaluating the results of comet assays. The authors present most of the findings to be statistically significant at $p < 0.0001$, including an increase in tail intensity,

however the biological significance of the magnitude of the change observed in tail intensity is questionable.

Marques, M.F.C. (1999). A Micronucleus Study in Mice for Glifosate Tecnico Nufarm. Bioagri Laboratarios Ltda. Study No: RF-G12.79/99. December 27, 1999. MRID 49957412. Unpublished.

In a bone marrow micronucleus assay, 5 adult male and female per dose Swiss Albino mice were treated with glyphosate (95% purity) with two intraperitoneal injections of 187.5, 375 and 562.5 mg/kg were administered 24 hours apart to male and female mice. The animals were sacrificed 24 hours after the last injection. A negative control was dosed with an equivalent volume of the vehicle. Bone marrow cells were harvested at 24 hours following dosing and scored for micronucleated polychromatic erythrocytes (MPCEs) and the ratio of polychromatic to normochromatic erythrocytes (PCE: NCE). Cyclophosphamide (25 mg/kg) served as the positive control (5 mice).

The LD50 for glyphosate was 750 mg/kg. In the main study, there were no premature deaths or clinical signs observed in any of the dose groups. There were no effect of glyphosate technical on micronucleus formation on mice bone marrow erythrocytes when compared to the control. The positive control responded appropriately. There was no significant increase in the frequency of MPCEs in any treatment group at either harvest time

Matsumoto (1995). HR-001: In vitro cytogenetics test. The Institute of Environmental Toxicology, Tokyo, Japan. Laboratory Project ID: IET 94-0143. May 29, 1995. MRID 50017106. Unpublished.

In an in vitro cytogenetics test to evaluate the clastogenic potential of glyphosate in Chinese hamster lung (CHL) cells, cells were treated with glyphosate (94.68% purity) at concentrations of 125, 250, 500 and 1000 μg/mL for 24 hours (-S9) or 62.5, 125, 250 and 500 μg/mL for 48 hours (-S9). CHL cells were also treated in the presence of S9 for 6 hours at concentrations of 250, 500, and 1000 μg/mL. The study was conducted according to OECD guideline 473. The source of the S9 was from the liver of Sprague Dawley rats induced with phenobarbital. The highest test concentrations were selected based on preliminary toxicity tests. Colchicine was added to the cultures 2 h prior to harvesting to arrest metaphase. A total of 200 metaphases were evaluated for each dose (100 metaphase cells/culture). Mitomycin C (-S9) and benzo(a)pyrene (+S9) served A decrease in pH as indicated by a change in the color of the medium was reported at ≥ 500 μg/mL glyphosate. No significant increases in the frequency of cells with chromosomal aberrations were observed at any test concentration or time point in the presence or absence of S9 activation. The positive controls induced the appropriate responses in this test system.

Mladinic, M., et al., (2009a). Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro. Environ Mol Mutagen 50(9): 800-807.

In this literature study, the genotoxic and oxidative potential of glyphosate was evaluated in human lymphocytes in vitro. The alkaline comet assay and in vitro micronucleus (with FISH analysis) assay were used to evaluate potential genotoxicity. Ferric-reducing ability of plasma (FRAP), thiobarbituric acid reactive substances (TBARS) and the hOGG1 modified comet assay were used to measure glyphosate's oxidative potential. The authors state that the treatment was performed in accordance with OECD chemical testing guidelines. Blood samples were taken from three healthy male donors. For each donor, duplicate cultures were treated 0.5, 2.91, 3.5, 92.8 and 580 μg/mL glyphosate (98% purity) adjusted to a pH of 7.2. Each concentration was treated for 4 h with and without S9 activation. For the micronucleus assay, following exposure to glyphosate, the cells were washed, treated with phytohemagglutinin and cultured for an additional 72 h. Human liver S9 mix was used for metabolic activation. Ethyl methanesulfonate and cyclophosphamide served as the positive controls.

In the alkaline comet assay, the authors reported a statistically significant increase in tail intensity at 92.8 μg/mL and increase in tail intensity and tail length at 580 μg/mL in the absence of S9. With S9, a statistically significant increase in tail intensity was reported only at 580 μg/mL with increased tail length at 3.5 μg/mL and above. Using the hOGG1 comet assay, The only statistically significant increase in tail intensity or tail length versus the negative control was at 3.5 μg/mL; however, the response was not seen at higher concentrations and was not concentration related.

In the micronucleus assay, no increase in micronucleated cells were seen at any concentration in the absence of S9. In the presence of S9, a statistically significant increase in the frequency of micronucleated cells was reported only at 580 μg/mL. Nuclear buds and nucleoplasmic bridges were slightly increased at concentrations of 3.5 μg/mL and above.

Statistically significant increases in FRAP and TBARS values were reported only at 580 lg/ml regardless of metabolic activation.

The authors conclude that since no clear dose dependent effects were observed for any of the assays, that glyphosate at concentrations relevant to human exposure do not post a significant health risk.

Reviewer comments: The exposure protocol that is different from OECD recommendations for the MN assay. OECD recommends that whole blood or separated lymphocytes are cultured in the presence of a mitogen (e.g. PHA) prior to exposure to the test substance. However, the authors exposed the cells to glyphosate for 4 hours, washed the cells and then cultured the cells into culture in the presence of PHA to stimulate growth. If glyphosate induces MN by an aneugenic mechanism, this effect very likely would not have been detected with the protocol described in this paper. Additionally, cells would have undergone more than one mitosis at72 h after exposure when the cells were harvested. Furthermore, a slide-based method was used for analysis of micronuclei and the authors did not report being blind to treatment. Because of these limitations, caution should be used with interpreting the results from this study.

Mladinic, M. et al., (2009b). Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay. Toxicology Letters 180: S170-S171.

In this published literature study, the clastogenic and aneugenic potential of glyphosate, terbuthylazine and carbofuran was evaluated in human lymphocytes using an in vitro micronucleus assay with fluorescence in situ hybridization (FISH) using centromeric DNA probes. This review focuses only on the findings for glyphosate. Human lymphocytes from three donors were cultured and exposed to 0.5, 2.91, 3.5, 92.8 and 580 µg/mL glyphosate (98% purity) adjusted to a pH of 7.2. Each concentration was treated for 4 h with and without S9 activation. Following exposure to glyphosate, the cells were washed, treated with phytohemagglutinin and cultured for an additional 72 h. Human liver S9 mix was used for metabolic activation. Ethyl methanesulfonate and cyclophosphamide served as the positive controls.

No statistically significant increases in frequency of cells with micronuclei (MN) were reported at any concentration in the absence of S9. In the presence of S9, a statistically significant ($p<0.01$) increase in the frequency of MN was seen only at 580 µg/mL. Increase in nuclear buds and nucleoplasmic bridges were seen only at 580 µg/mL. No concentration related increases in centromere or DAPI signals were observed with glyphosate treatment.

Reviewer comments: The authors attempted to determine whether glyphosate and other pesticides induced MN, and if so, whether they arose from a clastogenic mechanism or an aneugenic mechanism by staining for the presence of centromeres in micronuclei via fluorescence in situ hybridization (FISH). However, the authors exposed the cells to glyphosate for 4 hours, washed the cells and then cultured the cells into culture in the presence of PHA to stimulate growth. If glyphosate induces MN by an aneugenic mechanism, this effect very likely would not have been detected with the protocol described in this paper since glyphosate would not have been present to disrupt mitotic spindle machinery, an event that can lead to chromosome loss. .Additionally, cells would have undergone more than one mitosis at 72 h after exposure when the cells were harvested. Due to these limitations, caution should be taken when interpreting the findings in this study.

Moriya, M. et al. 1983. Further mutagenicity studies on pesticides in bacterial reverse assay systems. Mutation Research, 116 (1983), 185-216.

In a published literature study, 228 pesticides (including glyphosate) were tested in a bacterial reverse mutation assay system using *Salmonella typhimurium* strains TA100, TA98, TA1535, TA1537 and TA1538 and *Escherichia coli* staring WP2 *hcr*. This summary focuses on the results for glyphosate only. The article states that all pesticides were tested up to 5000 µg/plate with and without S9 activation unless toxicity was observed using the plate incorporation method. In cases where a pesticide showed mutagenicity in the concentration range of 1000-5000 µg/plate, the compound was tested concentrations more than 5000 µg/plate to evaluate a potential dose-relationship. For pesticides that tested positive in one or more strains, additional analyses were reported including comparisons for chemical classes and structures and estimation

of mutagenic potencies. In the case of glyphosate, the authors report negative findings for strains tested. No further analyses were conducted for this pesticide.

NTP, 1992. NTP Technical Report on Toxicity Studies of Glyphosate. National Toxicology Program Toxicity Reports Series 16. NIH Publication 92-3135, July 1992. U.S. Department of Health and Human Services. Published.

In two independent trials of a reverse mutation assay in bacteria *Salmonella typhimurium* strains TA1535, TA97, TA98, and TA100 were exposed to glyphosate; 99% purity in water at concentrations of 10, 33, 100, 333, 1000, 3333 and 10000 µg/plate in the preincubation assay. The tests were conducted with and without S9 activation with included 10% and 30% S9 from Aroclor 1254-induced male Syrian hamster liver and male Sprague Dawley rat liver. Standard strain-specific mutagens served as positive controls.

Evidence of slight toxicity was reported at 3333 and 100000 µg/plate. No increase in revertant colonies was observed at any test concentration in the presence or absence of S9 activation. The positive controls induced the appropriate response in all strains in the presence and absence of S9-activation. Glyphosate acid is therefore, not mutagenic in this test system.

NTP also conducted a peripheral blood micronucleus test at the termination of a 13 week feeding study. Male and female mice were administered glyphosate (99% pure) in the diet at doses of 205/213, 410/421, 811/844, 1678/1690 and 3393/3393 mg/kg/day in males/females, respectively. At study termination, blood smears were prepared from blood samples collected via cardiac puncture from control and glyphosate treated animals. 10,000 normochromatic erythrocytes were scored for each animal. No increase in micronuclei was observed at any dietary concentration of glyphosate in either sex.

Piesova, E (2004). The influence of different treatment length on the induction of micronuclei in bovine lymphocytes after exposure to glyphosate. Folia Veterinaria. 48 (3): 130-134.

In a published literature study, the author evaluated the genotoxic and cytogenetic effects of glyphosate in bovine lymphocytes using an in vitro micronucleus assay using a 24 and 48 h treatment protocol. Whole blood human lymphocytes cultures were established from two donors. Glyphosate (62% purity) was added at 28, 56, 140, 280 and 560 µM to the cultures 24 and 48 h after they were initiated (treatment time was 48 and 24 h, respectively). Glyphosate was present in the lymphocyte cultures until the end of cultivation at 72 h. Cells were evaluated only in the absence of metabolic activation (i.e. -S9). Cytochalasin B was added to the cultures at 44 h post-initiation. Mitomycin C served as the positive control.

The author reports no statistically significant increases in micronuclei (MN) at 24 hours in other donor. At 48 hours, a significant increase in the MN induction was seen in one donor (donor A) at 280 µM, but not at 560 µM and only at 560 µM in the other donor (donor B).

The authors also reported that treatments with glyphosate for 48 h induced simultaneously decreases in the percentages of mononucleated cells and increased percentages of bi-, tri- and tetranucleated cells in comparison with treatments lasting only 24 h.

Reviewer comments. The findings in this study were negative following 24 h exposure to glyphosate and equivocal at 48 h. There is no indication that pH was monitored or adjusted in this assay. Glyphosate has been shown to decrease the pH of some culture media resulting in false positives in genotoxicity assays. Furthermore, there is no indication that the samples were coded in either assay and scorers were blind to treatment. These limitations may impact the interpretation and significance of the findings.

Piesova, E. (2005). The effect of glyphosate on the frequency of micronuclei in bovine lymphocytes in vitro. Acta Veterinaria-Beograd 55(2-3): 101-109.

In a published literature study, the author evaluated the cytogenetic effects of glyphosate in bovine lymphocytes using an in vitro micronucleus assay. Whole blood human lymphocytes cultures were established from two donors. Glyphosate (62% purity) was added at 28, 56, 140, 280 and 560 μM to the cultures 24 h after stimulation with phytohemagglutinin. The highest dose of glyphosate was chosen on the basis of the reduction in mitotic index by >50% The treatment period was 2 h with and without S9, or 48 h in the absence of S9. The S9 was derived from the liver of rats induced with Aroclor 1245. Cytochalasin B was added to the cultures at 44 h post-initiation. Mitomycin C (-S9) and cyclophosphamide (+S9) served as the positive controls.

The authors reported a statistically significant ($p<0.05$) increase in the frequency of micronuclei in one donor at 280 μM at 48 h (-S9), but not at 580 μM; and at 580 μM only in the second donor. In the presence of S9, no statistically significant increases in the in the frequency of micronuclei were seen in either donor. The positive controls induced the appropriate responses.

Reviewer comments: The findings in this study were negative following 24 h exposure to glyphosate and equivocal at 48 h. There is no indication that pH was monitored or adjusted in this assay. Glyphosate has been shown to decrease the pH of some culture media resulting in false positives in genotoxicity assays. Furthermore, there is no indication that the samples were coded in either assay and scorers were blind to treatment. These limitations may impact the interpretation and significance of the findings.

Peluso M. et al. (1998). ^{32}P-Postlabeling detection of DNA adducts in mice treated with herbicide Roundup. Environ and Mol Mutagen 31:55-59.

In this published article, the ^{32}P-DNA post-labeling technique was used to determine if glyphosate technical or the glyphosate formulation Roundup can induce DNA adducts in the kidney and liver of mice. This review focuses only on the findings for glyphosate technical. Swiss CD1 male and female mice were administered 130 or 270 mg/kg glyphosate (purity not reported) by i.p injection. Untreated mice were used as negative controls. Animals were

sacrificed 24 h and the DNA was isolated from the liver and kidney, treated with nuclease P1 and the hydrolyzed-enriched adducted nucleotides were ^{32}P labeled and resolved on thin layer chromotography plates. Chromatograms were visualized by autoradiography and the level of adducts were determined by excising the areas of the chromatograms and measure the level of radioactivity. No DNA adducts were observed in the kidney or liver of mice treated with glyphosate technical.

Rank J., et al. (1993). Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using bone marrow micronucleus test, Salmonella mutagenicity test, and Allium anaphase-telophase test. Mut Res. 300: 29-36.

In a bone marrow micronucleus assay, groups 5 adult male and female NMRI-Bon mice were i.p. injected with 150 or 200 mg/kg glyphosate isopropylamine (purity not specified). Bone marrow cells were harvested at 24 and 48 h. 1000 polychromatic erythrocytes (PCEs) were examined to determine the frequency of micronucleated PCEs.

There were no sex differences in response to glyphosate treatment. No statistically significant increases in the frequency of micronucleated PCEs was seen at either dose. The positive control induced an appropriate response.

Ribeiro do Val, R. (2007). Bacterial reverse mutation test (Ames test) for Glifosato Technico Helm. TECAM Tecnologia Ambiental Ltda., Sao Paulo, Brasil. Study Number 3393/2007-2.0AM, Report Number RL3393/2007-2.0 AM-B, December 13, 2007. MRID 50000903. Unpublished.

In a reverse mutation assay, bacteria *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and TA102 were exposed to glyphosate (98.01% purity in water) at concentrations of 648, 1080, 1800, 3000 and 5000 µg/plate with and without S9 activation using the plate incorporation method according to OECD guideline 471. The S9 fraction, purchased from Moltox (Annapolis, MD), contained the microsomal fraction of rat liver induced with Aroclor 1254. A preliminary range finding assay was conducted with the TA100 strain at concentrations 8, 40, 200, 1000, and 5000 µg/plate glyphosate to select concentrations for the definitive test.

No toxicity was reported in the range finding test. In the definitive test, no significant increases in the number of revertants at any test concentration in any strain was observed. The positive controls induced the appropriate response in all strains in the presence and absence of S9-activation. Glyphosate is negative in this test system.

Rouston, A., et al. (2014). Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation. Chemosphere 108: 93-100.

In a literature study, the in vitro micronucleus assay was conducted using the Chinese hamster ovary cell line (CHO-K1) to assess the potential photo-inducible cytogenetic toxicity of glyphosate, atrazine, aminomethyl phosphoric acid (AMPA), desethyl-atrazine and various mixtures. This review focuses on the findings for glyphosate technical (alone) only. CHO-K1 cells were treated with 5, 10, 50 and 100 µg/mL glyphosate (purity not provided) for 3 h with and without S9 activation. The cells were rinsed and then treated with cytochalasin B for an additional 24 h, prior to fixation and preparation of slides. Cytokinesis blocked proliferation index (CBPI) was used as a measure to assess cytotoxicity to select the adequate concentrations for micronuclei evaluation. A total of 2000 binucleated cells were examined for each test concentration. S9 was prepared from the liver of male Sprague Dawley rats induced with Aroclor 1254. Mitomycin C and benzo(a)pyrene served as the positive controls with and without S9, respectively

No statistically significant increases in the percent of binucleated cells with micronuclei (BMC) were reported for cells treated with glyphosate in the absence of S9 activation. In the presence of S9, the authors reported statistically significant ($p<0.001$) increase in percent BMCs at 10, 50 and 100 µg/mL. The percent BMCs were similar (39 ± 2.8, 41 ± 4.2, and 42.5 ± 2.8 for 10, 50 and 100 µg/mL groups, respectively) at concentrations where statistically significant increases were noted compared to the negative controls (the % BMC for the controls was 9.5 ± 0.7).

Shirasu (1978). Microbial mutagenicity. Institute of Environmental Toxicology, Tokyo, Japan. July 20, 1978. MRID 00078619. Unpublished.

In a Rec- assay conducted for regulatory purposes, two strains of *Bacillus subtillus*, recombinant wild (H17) and deficient (M45), were thawed and streaked separately onto an agar plate. A filter paper disk was soaked with 0.2 ml of 10 mg/ml aqueous solution of glyphosate (98.4% purity), and placed so as to cover the starting parts of the bacterial streaks. After an overnight incubation, the length of the inhibitory zone of each streak was measured. Kanamycin served as the negative control and mitomycin C served as the positive control. No inhibitory zone was noted in either strain at doses of 20-2000 µg test material/disk. The controls responded appropriately.

Reverse Mutation Assay: In two independent trials of a reverse mutation assay in bacteria *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* WP2 *uvrA* were exposed glyphosate (98.4% purity) in water at concentrations of 10, 50, 100, 500, 1000 and 5000 µg/plate (+/- S9 activation). The S9 was derived from Aroclor 1254-induced rat livers. Standard strain-specific mutagens served as positive controls. There were no appreciable increase in the mean number of revertants/plate in any strain in both trials. The positive controls induced the appropriate response in all strains in the presence and absence of S9-activation. Glyphosate is therefore, not mutagenic in this test system.

Sivikova, K and Dianovksy, J. (2006). Cytogenetic effect of technical glyphosate on cultivated bovine peripheral lymphocytes. International Journal of Hygiene and Environmental Health. 209: 15-20.

In this published literature study, glyphosate isopropylamine salt (purity 62%) was evaluated for its potential to induce chromosome aberrations (CA) and sister chromatid exchanges (SCE) in cultured bovine peripheral lymphocytes in vitro. Lymphocyte cultures were exposed to glyphosate at 28, 56, 140, 280, 560 or 1120 µM. It was stated that the highest test concentration was selected to reduce the mitotic index by more than 50%. Ethyl methanesulfonate and mitomycin C served as the positive controls. The S9 fraction was obtained from the liver of mice induced with Aroclor 1254. Whole blood from two healthy young bulls was used to establish the lymphocyte cultures. Lymphocyte cultures were stimulated with phytohemagglutinin prior to treatment and cultures were treated with colchicine 2 h before harvest. Cultures without S9 activation were treated with glyphosate for 24 h (CA and SCE) or 48 h (SCE), while cultures with S9 activation were treated with glyphosate for 2 h (SCE). For the SCE assay, bromodeoxyuridine was added to the culture 24 h after initiation of cell division. For CA, 100 metaphases were analyzed and the mitotic index was evaluated by scoring the metaphases of 2000 cells. Fifty metaphase per donor and test concentration were evaluated for SCE.

No statistically significant increases in CAs were reported at any test concentration in either donor at 24 h. Significant decreases in mitotic indices were observed at ≥560 µM glyphosate. The authors reported statistically significant increase in SCEs at concentrations of ≥ 56 µM in both donors at 24 h and in one donor at ≥ 289 µM and at 560 µM in the other donor at 48 h. In cultures exposed for 2 h with S9, statistically significant. However there was no clear dose response for the effect across the concentration range tested. The highest concentrations (560 and 1120 µM) resulted in a reduction of mitotic and proliferation indices. In the 2 h-assay with S9 a statistically significant frequency of SCE was observed only in cultures treated with the agent at a concentration of 140 µM, but not at the higher concentration of 280. The positive controls induced the appropriate responses.

Reviewer comments: It is unclear from the information provided in the article why glyphosate with a purity of only 62% was used in this study. The increases in SCEs observed in this study did not show a clear concentration related increase across a 40-fold increase in the concentrations tested.

Sokolowski, A. (2007a). *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Glyphosate Technical (NUP-05068). RCC Cytotest Cell Research GmbH, Rossdorf, Germany. Study Number 1061401. March, 16, 2007. MRID 49957406. Unpublished.

In two independent trials of a reverse mutation assay, bacteria *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* WP2 *uvrA* were exposed to glyphosate; 95.1 % purity in water at concentrations of 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate +/- S9 mix using the plate incorporation method and 33, 100, 333, 1000, 2500 and 5000 µg/plate +/- S9 mix using the pre-incubation method. The S9 fraction was derived from the livers of Wistar male rats induced with phenobarbital/ ß-napthoflavone. Standard strain-specific mutagens served as positive controls.

No evidence of toxicity was observed in the plate incorporation test. Some evidence of toxicity was seen in the TA157 strain at 5000 µg/plate without metabolic activation using the pre-incubation method (4 vs. 11 in the control group). No significant or dose-related increases in the number of revertant colonies were observed in any of the strains exposed to glyphosate with or without metabolic activation. The positive controls induced the appropriate response in all strains in the presence and absence of S9-mix. Glyphosate is considered to be negative in this test system.

Sokolowski, A. (2007b). *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Glyphosate Technical (NUP-05070). RCC Cytotest Cell Research GmbH, Rossdorf, Germany. Study Number 1061402. March, 16, 2007. MRID 49957407. Unpublished.

In two independent trials of a reverse mutation assay, bacteria *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* WP2 *uvrA* were exposed to glyphosate; 95.0 % purity in water at concentrations of 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate +/- S9 mix using the plate incorporation method and 33, 100, 333, 1000, 2500 and 5000 µg/plate +/- S9 mix using the pre-incubation method. The S9 fraction was derived from the livers of Wistar male rats induced with phenobarbital/ ß-napthoflavone. Standard strain-specific mutagens served as positive controls.

Some evidence of toxicity (reduced number of revertant colonies) was reported in *E. coli* treated with 5000 µg/plate in the absence of metabolic activation in the plate incorporation test and in TA98 at 5000 µg/plate with metabolic activation using the pre-incubation method. No significant or dose-related increases in the number of revertant colonies were observed in any of the strains exposed to glyphosate with or without metabolic activation. The positive controls induced the appropriate response in all strains in the presence and absence of S9-mix. Glyphosate is considered to be negative in this test system.

Sokolowski, A. (2007c). *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Glyphosate Technical (NUP-05067). RCC Cytotest Cell Research GmbH, Rossdorf, Germany. Study Number 1061403. March, 16, 2007. MRID 49957408. Unpublished.

In two separate trials of a reverse mutation assay in bacteria *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* WP2 *uvrA* were exposed to glyphosate; 95.0 % purity in water at concentrations of 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate +/- S9 mix using the plate incorporation method and 33, 100, 333, 1000, 2500 and 5000 µg/plate +/- S9 mix using the pre-incubation method. The S9 fraction was derived from the livers of Wistar male rats induced with phenobarbital/ ß-napthoflavone. Standard strain-specific mutagens served as positive controls.

Some evidence of toxicity (reduced growth) was reported in *E. coli* treated with 5000 µg/plate in the absence of metabolic activation only. No significant or dose-related increases in the number of revertant colonies were observed in any of the strains exposed to glyphosate with or without

metabolic activation. The positive controls induced the appropriate response in all strains in the presence and absence of S9-mix. Glyphosate is considered to be negative in this test system.

Sokolowski, A. (2009b). Glyphosate technical- Salmonella Typhimurium and Escherichia Coli reverse mutation assay. Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, German. Study and Report Number 1264500. December 18, 2009. MRID 49961801. Unpublished.

In two separate trials of a reverse mutation assay in bacteria *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* WP2 *uvrA* pKM 101 and WP2 pKM 101 were exposed to glyphosate; 96.3 % purity in water at concentrations of 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate (+/- S9 activation). The first trial was conducted with all tester strains using the plate incorporation assay followed by a second trial using the preincubation method. The S9 fraction was derived from the livers of Wistar male rats induced with phenobarbital/ ß-napthoflavone. Standard strain-specific mutagens served as positive controls.

No toxic effects were seen at any test concentration. No substantial increase (statistically significant or >2-fold compared to negative controls) in the mean number of revertant colonies was observed in any strain at any test concentration of glyphosate. The positive controls induced the appropriate response in all strains in the presence and absence of S9-activation. Glyphosate is considered to be negative in this test system.

Sokolowski, A. (2010). *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Glyphosate TC spiked with glyphosine. RCC Cytotest Cell Research GmbH, Rossdorf, Germany. Study Number 1332300. April 7, 2010. MRID 500000902. Unpublished.

In two separate trials of a reverse mutation assay in bacteria *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* WP2 *uvrA* were exposed to glyphosate technical grade (97.16% purity) spiked with 0.63% (w/w) glyphosine in water at concentrations of 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate +/- S9 mix using the plate incorporation method (Experiment 1) and the pre-incubation method (Experiment 2). The S9 fraction was derived from the livers of Wistar male rats induced with phenobarbital/ ß-napthoflavone. Standard strain-specific mutagens served as positive controls.

No evidence of toxicity was reported up to 5000 µg/plate +/- S9 mix. No substantial increase (statistically significant or >2-fold compared to negative controls) in the mean number of revertant colonies was observed in any strain at any test concentration of glyphosate. The positive controls induced the appropriate response in all strains in the presence and absence of S9-activation. Glyphosate (spiked with glyphosine) is considered to be negative in this test system.

Suresh, T.P. (1992). Dominant Lethal Test in Wistar Rats: Glyphosate Technical. Rallis Agrochemical Research Station, Bangalore, India. Study No. TOXI: 888-DLT. November 3, 1992. MRID 49987404. Unpublished.

In a dominant lethal test conducted to determine the mutagenic potential of glyphosate to induce genetic damage in germ cells and cause embryonic or fetal death. Glyphosate (96.8% purity) in peanut oil was administered by oral gavage to 5 male Wistar rats at 200, 1000 or 5000 mg/kg in a single dose. The treated and vehicle control males were mated with virgin females every week for 10 weeks. The fertility indices were determined each week after day 16 of co-habitation. Ethyl methane Sulphonate was used as the positive control.

Glyphosate did not induce a dominant lethal effect at any test concentrations. The changes seen in the fertility indices were inconsistent and not related to the dose of glyphosate. The positive control induced the appropriate response in this test system.

Suresh, T.P. (1993b). Mutagenicity-Micronucleus Test In Swiss Albino Mice. Rallis India Limited. Study No: TOXI: 889-MUT.MN. May 6, 1993. MRID 49987407. Unpublished.

In a bone marrow micronucleus assay, 5 adult male and female Swiss Albino mice per dose were treated once orally with glyphosate (96.8% purity) in peanut oil using single oral doses of 0, 50, 500 and 5000 mg/kg (10 mice per sex in the vehicle control). Bone marrow cells were harvested at 24 hours following dosing and scored for micronucleated polychromatic erythrocytes (MPCEs) and the ratio of polychromatic to normochromatic erythrocytes (PCE: NCE). Cyclophosphamide (100 mg/kg) served as the positive control (5 mice).

At the doses tested there were no compound and dose related clinical signs of toxicity and mortality in the treatment groups. There were marginal decreases in absolute body weights in the mid- and high-dose groups, and the positive control group. There were no effect of glyphosate technical on micronucleus formation on mice bone marrow erythrocytes when compared to the control in males. In females, the incidence of micronucleated PCE and total RBCS were significantly higher ($P \leq 0.05$) than in the control. The positive control responded appropriately. There was no significant increase in the frequency of MPCEs in any treatment group at harvest time.

Suresh, T.P. (1994). Genetic Toxicology- In vivo mammalian bone marrow cytogenetic test-Chromosomal analysis. Rallis Agrochemical Research Station, Bangalore, India. January 1, 1994. MRID 49987408. Unpublished.

In an in vivo chromosomal aberration assay, glyphosate (96.8% purity) in groundnut oil was administered by oral gavage to 5 male and 5 female Swiss albino mice at doses of 50, 500 and 5000 mg/kg for two consecutive days. Twenty four hours after the second dose, animals were administered colchicine by i.p. injection, and animals were sacrificed 90 minutes later to obtain bone marrow suspensions. Bone marrow cells were harvested, fixed and stained. The mitotic index was estimated by counting the number or metaphases per 100 blast cells per slide. Slides were screened for 50 analyzable metaphases per animal. Cyclophosphamide served as the positive control.

There were no clinical signs of toxicity in the study. No statistically significant increases in chromosomal aberrations were observed at doses up to 5000 mg/kg. The positive control produced the appropriate response.

Thompson, P.W. (1996). Technical Glyphosate: Reverse Mutation Assay "Ames Test" Using *Salmonella typhimurium* and *Escherichia coli.* Safepharm Laboratories Limited, Derby, UK. Study Number 434/014. February 20, 1996. MRID 49957409. Unpublished.

In two separate trials of a reverse mutation assay in bacteria *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* WP2 *uvrA* were exposed to glyphosate; 95.3 % purity in water at concentrations of 50, 150, 500, 1500 and 5000 µg/plate (+/- S9 activation). A preliminary toxicity study was conducted with TA100 and WP2 *uvrA* strains with 50 – 5000 µg/plate glyphosate. The two main experiments were conducted using the direct plate incorporation method. The S9 fraction was derived from the livers of Sprague Dawley male rats induced with Aroclor 1254. Standard strain-specific mutagens served as positive controls.

In the preliminary study, evidence of toxicity (reduction of revertant colonies) was seen at 5000 µg/plate in TA100 (12 colonies versus 69 in the control group). No toxicity was seen in the bacterial strain WP2 *uvrA* at any concentration. No significant increase in the mean number of revertant colonies compared to the controls was observed in any strain at any test concentration of glyphosate with or without S9-activation. The positive controls induced the appropriate response in all strains in the presence and absence of S9-activation. Glyphosate is considered to be negative in this test system.

Wilderman, A.G. and Nazar, R.N. (1982). Significance of plant metabolism in the mutagenicity and toxicity of pesticides. Canadian Journal of Genetics and Cytology 24(4): 437-449.

In a published literature study, the authors investigated the influence of plant metabolism on mutagenesis using a Salmonella bioassay in the presence and absence of both rat S9 and plant homogenates. The 5 tester strains used in this study included *Salmonella typhimurium* TA98, TA100 TA1535 TA1537 and TA1538. The S9 supernatant was prepared from the liver of rats induced with Aroclor 1254. Plant homogenates prepared from 5-day old seedlings of corn and wheat were used to evaluate the plant metabolic effects on pesticide mutagenicity. . Standard mutagens (2-nitrofluorene, sodium azide and 2-aminoanthracene) served as positive controls. Fifty pesticides were evaluated in this paper; however, this summary will focus only on the findings for glyphosate. In this study glyphosate was tested at a single concentration of 25 µg/plate. Glyphosate did not induce a significant increase in revertants in the presence or absence of S9-mix or cell-free plant homogenates.

Wright, N.P. (1996). Technical Glyphosate: Chromosomal aberration test in CHL cells in vitro. Safepharm Laboratories Limited, Derby, UK. Study Number 434/015. March 13, 1996, MRID 49957410. Unpublished.

In a mammalian cell cytogenetics (chromosome aberration) assay Chinese hamster lung (CHL) cultures were exposed to glyphosate (95.3%) in culture medium at concentrations from 39.1 to 1250 µg/mL for 6 hours with and without S9 metabolic activation, and for 24 and 48-hours of continuous exposure without S9. The S9 fraction was derived from male Sprague Dawley rats induced with Aroclor 1254. Mitomycin C and cyclophosphamide served as positive controls in the absence and presence of S9, respectively. A preliminary toxicity test was performed at concentrations up to 5000 µg/mL with and without metabolic activation to determine the appropriate concentrations for the definitive test.

The preliminary toxicity test revealed a significant reduction in cell number (49% of control) at 6 hours (-S9) and there were not metaphases that could be scored at 5000 µg/mL following 24 and 48 hours of continuous exposure (-S9). Additionally, an evaluation of the culture medium revealed a significant reduction in pH (≥ 1 pH unit) at 2500 and 5000 µg/mL with and without S9 activation. Therefore the maximum concentration with appropriate culture conditions was determined to be 1250 µg/mL. The negative (vehicle) control cultures responded with chromosomal aberration values within the expected range. Glyphosate did not induce a statistically significant or concentration-related increase in the frequency of cells with chromosomal aberrations at any test concentration or exposure period with or without S9 activation.

Zaccaria, C.B. (1996). A micronucleus study in mice for the product Glyphosate. BioAgri. Study No: G.1.2 – 06/96. November 18, 1996. MRID 49961501. Unpublished.

In a bone marrow micronucleus assay, 5 adult male and female Swiss Albino mice per dose were dosed twice through intraperitoneal injection with glyphosate (96.8% purity) in water using single oral doses of 0, 68, 137, and 206 mg/kg (corresponding to 75%, 50% and 25% of the LD_{50}). Bone marrow cells were harvested at 24 hours following dosing and scored for micronucleated polychromatic erythrocytes (MPCEs) and the ratio of polychromatic to normochromatic erythrocytes (PCE: NCE). Cyclophosphamide (25 mg/kg) served as the positive control (5 mice).

The LD50 for glyphosate was 275 mg/kg. There were no premature deaths or clinical signs observed in any of the dose groups. There were no effect of glyphosate technical on the polychromatic and normochromatic erythrocytes ratio following treatment with glyphosate. A statistically significant increase in micronucleated polychromatic and normochromatic erythrocytes was observed in animals treated with cyclophosphamide. There was no significant increase in the frequency of MPCEs in any treatment group at harvest time.

Zoriki Hosomi, (1997). Mammalian Erythrocyte Micronucleus Test for Glifosato Tecnico Helm. TECAM Tecnologia Ambiental Sao Roque Ltd. Laboratory Project ID: RL 3393/2007-3.0MN-B. December 13, 2007. MRID 500000901. Unpublished.

In a bone marrow micronucleus assay, 6 adult male Swiss mice per dose were treated once via oral gavage with glyphosate (98% purity) in deionized water at doses of 0, 8, 15 and 30 mg/kg. Only males were employed since no information showing significant differences between males and female sensitivity and/or toxicity was available for the test substance. Animals were treated twice at 0 and 24 h (two treatments at 24 hours interval) and bone samples were taken approximately 24 hours following the final treatment. Bone samples were scored for micronucleated polychromatic erythrocytes (MPCEs) and the ratio of polychromatic to normochromatic erythrocytes (PCE: NCE). Cyclophosphamide (75 mg/kg) served as the positive control (5 mice).

There were no premature deaths or clinical signs seen in any of the dose groups. When animals treated with technical glyphosate were compared to the concurrent negative control group, no statistically increase in the number of micronuclei were observed in the low- and mid-dose groups tested. In the 30 mg/kg group, statistical significant results were obtained ($x^2 = 5.44$; P = 0.020), though this result had no biological relevance when compared to historical control and published data. The positive control showed a marked increase in the incidence of micronucleated polychromatic erythrocytes hence confirming the sensitivity of the system to the known mutagenic activity of cyclophosphamide under the conditions of the test.